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Naïve T-Cell Dynamics in Human Immunodeficiency Virus Type 1 Infection: Effects of Highly Active Antiretroviral Therapy Provide Insights into the Mechanisms of Naïve T-Cell Depletion

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Both naïve CD4+ and naïve CD8+ T cells are depleted in individuals with human immunodeficiency virus type 1 (HIV-1) infection by unknown mechanisms. Analysis of their dynamics prior to and after highly active antiretroviral therapy (HAART) could reveal possible mechanisms of depletion. Twenty patients were evaluated with immunophenotyping, intracellular Ki67 staining, T-cell receptor excision circle (TREC) quantitation in sorted CD4 and CD8 cells, and thymic computed tomography scans prior to and ~6 and ~18 months after initiation of HAART. Naïve T-cell proliferation decreased significantly during the first 6 months of therapy ($P < 0.01$) followed by a slower decline. Thymic indices did not change significantly over time. At baseline, naïve CD4+ T-cell numbers were lower than naïve CD8+ T-cell numbers; after HAART, a greater increase in naïve CD4+ T cells than naïve CD8+ T cells was observed. A greater relative change (n-fold) in the number of TREC+ T cells/μl than in naïve T-cell counts was observed at 6 months for both CD4+ (median relative change [n-fold] of 2.2 and 1.7, respectively; $P < 0.01$) and CD8+ T cell pools (1.4 and 1.2; $P < 0.01$). A more pronounced decrease in the proliferation than the disappearance rate of naïve T cells after HAART was observed in a second group of six HIV-1-infected patients studied by in vivo pulse labeling with bromodeoxyuridine. These observations are consistent with a mathematical model where the HIV-1-induced increase in proliferation of naïve T cells is mostly explained by a faster recruitment into memory cells.

The mechanisms leading to CD4+ T-cell depletion during human immunodeficiency virus (HIV) infection and CD4+ T-cell restoration during therapy with highly active antiretroviral therapy (HAART) remain undefined. Direct viral cytopathogenicity or redistribution of lymphocytes between lymphoid tissues and the circulation can account only in part for these changes (28, 32, 41). Characterizing lymphocyte turnover in HIV-infected patients can potentially lead to a better understanding of these mechanisms.

A number of studies utilizing indirect methods (2, 21, 31, 40, 48) and, more recently, direct methods using deuterated glucose (25, 36) or 5-bromo-2′-deoxyuridine (BrdU) (29) to measure CD4 cell dynamics have shown that CD4 cell turnover is increased during chronic HIV type 1 (HIV-1) infection. Moreover, while preliminary cross-sectional studies described an increase in CD4 cell turnover in patients following initiation of HAART, suggesting a defect in CD4 cell production secondary to HIV infection (12, 25), longitudinal studies have clearly demonstrated a rapid and persistent decrease in CD4 cell proliferation following initiation of HAART, suggesting that the increase in CD4 cell turnover itself may be an important pathogenic mechanism of CD4 depletion (21, 29, 36). While this increased turnover was initially postulated to represent a homeostatic response to CD4 depletion (11, 12), such a hypothesis is inconsistent with the rapid reduction in proliferation of CD4+ as well as CD8+ T cells after viral suppression with HAART, prior to normalization of CD4 cell numbers (2, 29, 31, 36). These observations led to alternative hypotheses proposing that either HIV-directed or nonspecific immune activation drives increased turnover. Moreover, based in part on studies demonstrating that levels of immune activation in T cells, especially CD8 cells, are independent predictors of CD4 depletion and disease progression, immune activation is currently felt by many investigators to play a direct role in HIV-associated CD4 depletion (17, 34, 43).

An increase in turnover has been demonstrated in naïve T cells (24, 26) as well as memory T cells during pathogenic lentiviral infection. Whereas memory CD4+ but not memory CD8+ T cells decrease in number during chronic HIV infection, both naïve CD4+ as well as naïve CD8+ T cells are depleted during such infection (5, 22, 39). This observation led to the conclusion that naïve T-cell depletion is one of the hallmarks of HIV infection. While infection of naïve T cells has been documented, this appears to be a relatively rare event that cannot quantitatively explain the loss of naïve CD4+ T cells. The observation that both naïve CD4+ and naïve CD8+ T cells decrease during HIV infection led to the hypothesis that persistent hyperactivation of the immune system leads to erosion

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TABLE 1. Baseline patient characteristics for group 1

Parameter	Value for group ($n = 20$)	
	Mean	Range
Age (yr)	37	29–49
HIV RNA (copies/ml)	174,875	4,500–1,602,790
CD4 (cells/μl)	282	6–687
Naïve CD4 (cells/μl)	83	0–264
CD8 (cells/μl)	819	34–1823
Naïve CD8 (cells/μl)	115	3–210

of naïve T cells by their increased recruitment into memory cells (20), probably through antigen- and nonantigen-specific stimulation, as has been shown in animal models (18, 37).

Because the thymus is the source of new T cells, examining thymic function during HIV infection and therapy is critical to studies of T-cell dynamics (14, 19). Due to the difficulties in directly studying thymic function, quantitation of T-cell receptor excision circles (TRECs) has been utilized as a surrogate of thymic function. Early studies measuring the number of TRECs per naïve T cell suggested that HIV infection leads to a decrease in thymic function and that improved thymic function contributes to immune reconstitution following HAART (9). More recently, studies using mathematical modeling showed that the observed changes in TREC content cannot be explained solely by changes in thymic function (21) or by redistribution of T lymphocytes from lymphoid tissues to the blood (32); the observed TREC dynamics were more consistent with changes in peripheral proliferation and disappearance rates of T-lymphocytes.

In the current study we undertook a detailed examination of the relationship between T-cell turnover, thymic function, and immune activation in HIV-1-infected patients to better understand the contribution of these various parameters to the immunologic changes seen during HIV infection and therapy.

MATERIALS AND METHODS

Patient characteristics. Twenty-three HIV-1-infected patients who were initiating HAART ($n = 20$), starting HAART after a 6- and 24-month treatment interruption ($n = 2$), or switching from an ineffective to an effective HAART regimen ($n = 1$) were studied. Twenty previously untreated (or with limited nucleoside analogue exposure) HIV-1-infected patients (group 1) enrolled between 1997 and 2000 in a study to identify viral reservoirs in patients receiving antiretroviral therapy. These patients initiated therapy with indinavir, nevirapine, lamivudine, and zidovudine (or stavudine [one patient]). Drug changes were permitted for intolerance; for 14 patients, zidovudine was replaced by stavudine, and for 6 patients, indinavir was replaced by nelfinavir. After at least 1 year on study, additional changes were permitted at the patient's and referring physician's discretion; seven patients made such changes, usually for simplification of the regimen. All patients ultimately demonstrated a virologic response, with declines in plasma viral loads to levels below branched DNA assay limits (<50

copies/ml, 18 patients; <500 copies/ml, 2 patients) and all but 2 patients had an increase in CD4 cell numbers of at least 100 cells/mm³. Group 1 patients had immunophenotyping analysis and evaluation of thymic function by TREC analysis and thymic computed tomography (CT) scans at the following time points: 0 (pre-HAART), 1 (5 to 8 months, median of 6 months after start of therapy), and 2 (13 to 42 months, median of 18 months after start of therapy). Naïve cell labeling kinetics were analyzed for six HIV-1-infected patients (group 2) who had undergone in vivo pulse labeling with BrdU prior to and 3 to 6 months after the initiation of an effective HAART regimen. Three of these patients are also included in group 1; the remaining three received stavudine, lamivudine, indinavir, and nevirapine (one patient); stavudine, lamivudine, and efavirenz (1 patient); and abacavir, amprenavir, nelfinavir, and efavirenz (one patient). Results for CD4 and CD8 cell labeling, but not naïve cell labeling, have been previously reported for this group (29). Baseline characteristics for group 1 and pre- and post-HAART parameters for group 2 are shown in Tables 1 and 2.

Viral load. HIV-1 RNA levels were determined using a modification of the Roche Amplicor HIV Monitor assay kit (Indianapolis, IN) (27) or a branched DNA assay (Bayer Diagnostics, Tarrytown, NY) (3). Each assay had a lower limit of detection of 50 copies/ml; for two patients enrolled early in the study, the limit of detection was 500 copies/ml.

Immunophenotyping and intracellular staining for Ki67. Immunophenotypic analysis of cryopreserved peripheral blood mononuclear cells was performed using four-color immunofluorescence as previously described (42). Naïve cells were defined as CD45RO⁻ CD27⁺, central memory were defined as CD45RO⁺ CD27⁺, and effector memory were defined as CD45RO⁺ CD27⁻ for CD4 T cells and as CD27⁻ (CD45RO⁺ or CD45RO⁻) for CD8 T cells. Cells were stained intracellularly with Ki67-phycoerythrin (clone B56) or isotype (mouse immunoglobulin G1-phycoerythrin; clone MOPC-21) from BD/Pharmingen. T-cell proliferation was defined as the percentage of cells expressing Ki67 (15).

TREC determination. Signal joint TRECs (Sj-TRECs) in purified cell subsets were quantitated by real-time PCR by the cell lysis method as described previously (38). The consistency of the DNA content of the cell lysate was checked by real-time PCR using a ribosomal protein gene and a TaqMan gene expression assay kit from Applied Biosystems, Inc. (Foster City, CA). Because no more than one Sj-TREC can be produced per cell, the number of TRECs per unit volume of blood also represents the number of TREC⁺ T cells in the same unit volume.

Thymic CT scans. CT scans of the thymus were obtained prior to and at a median of 6 and 18 months after starting HAART. Scans were graded as previously described on a 0 (no thymic tissue) to 5 (thymic mass) scale by two independent radiologists blinded to clinical and laboratory results (35). In addition, computer-based density and volume analysis of the thymus was performed by transferring CT data to a GE Advantage Windows workstation (versions 2.1 and 4.0; GE Medical Systems, Advanced Windows Workstation Training Program, Milwaukee, WI). Contours of the anterior mediastinum were outlined by a radiologist-trained technician and corroborated by a radiologist (13).

BrdU infusion and flow cytometry. The fractions of BrdU⁺ CD4⁺ CD45RO⁻ and BrdU⁺ CD8⁺ CD45RO⁻ T cells were analyzed by flow cytometry as previously described (29).

Statistics. Changes in median values for each variable were tested for significance by the permutation test with paired samples computed with an exact method (44). Tests were performed using StatXact software. Association between variables was determined by the Spearman rank correlation test. Adjustment of P values for multiple testing was done by the Bonferroni method. Occasional data points were missing for group 1; however, all paired analyses were tested with n values that were ≥15.

The relative change (n-fold) of a variable H between time (t) points (e.g., t_0 and t_1) is defined as the ratio between the value of H at t_1 versus t_0: $H(t_1)/H(t_0)$.

TABLE 2. Pre- and post-HAART viral loads and CD4⁺ and CD8⁺ T cell counts for group 2

Patient no.	Age (yr)	HIV RNA (copies/ml)		CD4 (cells/μl)		CD8 (cells/μl)	
		Pre-HAART	Post-HAART	Pre-HAART	Post-HAART	Pre-HAART	Post-HAART
1	40	20,249	<50	169	174	686	488
2	48	56,663	<50	318	574	701	810
3	36	192,780	<50	442	734	842	783
4	40	348,778	892	280	384	1261	886
5	39	11,353	<50	888	1116	1142	1267
6	49	43,463	<50	134	216	939	914

Modeling. Differential equations were solved using Labview 7.0 (National Instruments, Austin, TX). The data were fitted to the differential equations using the Levenberg-Marquardt method (16).

Mathematical model for TREC analysis. To help interpret the data obtained in this study, we propose a slight generalization of a mathematical model originally described by Hazenberg et al. (21). The number of naïve T cells/µl (T) and the number of TREC$^+$ T cells/µl (T^+) are governed by the following equations:

$$\frac{dT}{dt} = \sigma + pT - dT$$

$$\frac{dT^+}{dt} = f\sigma - dT^+ \tag{1}$$

Naïve CD4$^+$ or CD8$^+$ T cells, T, in the periphery receive a constant input from the thymus, σ, proliferate at rate p (day^{-1}) and disappear at a rate d (day^{-1}). TREC$^+$ T cells, T^+, appear at a lower rate of thymic production ($f\sigma$) and disappear from the same compartment as naïve T cells at a rate d. We assume that naïve T cells can proliferate without losing their naïve phenotype as has been reported (45–47). Since TRECs do not replicate during cell mitosis (9), proliferation of TREC$^+$ T cells decreases the fraction of TREC$^+$ T cells per naïve T cell (T^+/T).

Since changes in T and T^+ occur very slowly, the steady-state values obtained by equation 1 can be used to analyze how changes in the parameters (σ, d, p, and f) would affect changes in the number of naïve cells, T, the number of TREC$^+$ cells, T^+, and the fraction of TREC$^+$ T cells per naïve T cell (T^+/T):

$$T = \frac{\sigma}{d - p}$$

$$T^+ = \frac{f\sigma}{d} \tag{2}$$

$$\frac{T^+}{T} = \frac{f(d - p)}{d}$$

For the generalization in equation 1, we assume that the disappearance rate, d, is a composite of two different factors: $d = \delta_A + \delta_R$, where δ_A represents the death rate of naïve cells and δ_R is the rate of priming of naïve cells into memory cells (7, 23). We also assume that the increase in the proliferation rate of naïve T cells during chronic HIV-1 infection ($p \rightarrow p + \Omega$) is largely explained by the increase in the rate of priming of naïve T cells into memory cells ($\delta_R \rightarrow \delta_R + \Omega$). Thus, the generalized model has been simulated to predict the changes in naïve T-cell counts, TREC$^+$ T cells, and the fraction of TRECs per naïve T cell after HAART, when the perturbation of the quasi-steady state induced by the administration of HAART is described mathematically by the reduction of Ω.

Mathematical model for the kinetics of BrdU-labeled naïve T cells. To describe the in vivo kinetics of BrdU-labeled naïve T cells in the blood after a 30-min BrdU infusion, we used the following semiempirical equation (29):

$$\frac{dL}{dt} = s - d^*L \qquad t \leq \tau$$

$$\frac{dL}{dt} = -d^*L \qquad t > \tau \tag{3}$$

The pool of labeled cells in the blood, L, is refilled at a constant rate s until time τ, and labeled cells disappear from the pool of naïve T cells in the blood with a disappearance rate d^*. In developing this semiempirical model, it is assumed that lymphoid tissue serves as an effective source of labeled cells that are distributed to the blood until equilibrium is reached (time τ), at which point the effective source ceases to affect changes in the concentration of labeled cells (29). Because BrdU$^+$ chromosomes segregate independently into daughter cells, labeled cells that have divided will still be counted as BrdU$^+$ cells, as long as the intensity of BrdU in each cell is higher than the threshold of flow cytometric detection. In human lymphocytes we estimate that the BrdU intensity decreases below the detection threshold after two to three divisions (data not shown). Thus, for highly proliferating cells equation 3 is approximately similar to a model where d^* consists only of the disappearance rate of labeled cells (29). For more slowly proliferating cells, such as naïve T cells, a possible contribution of proliferation can be taken into account by replacing d^* with $d - p$, which is similar to an equation proposed by Debacq and colleagues (4). When d^* is small ($\ll 1$) the solution of equation 3 for $t \leq \tau$ is given by $L(t) = \frac{s}{d^*}(1 - e^{-d^*t}) \xrightarrow{d^* \to 0} st$, or the

fraction of labeled cells increases approximately linearly over time with a rate s. Thus, the solution of equation 3 used in this analysis is given by

$$L(t) = st \qquad\qquad t \leq \tau$$

$$L(t) = L(\tau)\,e^{-d^*(t-\tau)} \qquad t > \tau \tag{4}$$

Moreover, if the peak of labeling is reached at similar times between recipients, the value of the fraction of BrdU-labeled cells at the peak will correlate with s and, thus, with proliferation rates (4).

For BrdU labeling, naïve CD4 cells are defined as CD45RO$^-$, as additional markers were not utilized in these analyses. For CD4 cells, this is a good approximation of true naïve cells (6, 30).

RESULTS

Effect of HAART on viral load, T-cell counts, and T-cell proliferation. Sixteen of 20 group 1 patients had suppression of viral loads to <50 copies/ml at time point 1, and 18 of 20 had the same result at time point 2. Subjects had a mean CD4 count increase compared to a baseline of 144 cells/µl (range, 33 to 515 cells/µl) at time point 1 (~6 months) and 286 cells/µl (range, 17 to 643 cells/µl) at time point 2 (~18 months; $P < 0.01$) (Table 3).

Statistically significant increases in naïve CD4$^+$ T cells were observed at both time points 1 and 2 compared to baseline (from 87 to 127 and 164 cells/µl, respectively; $P < 0.01$), while statistically significant increases in naïve CD8$^+$ T cells were only observed at time point 2 (from 114 to 175 cells/µl; $P < 0.05$) (Table 3). At baseline, naïve CD4$^+$ T-cell numbers were lower than naïve CD8 T-cell numbers ($P < 0.05$) but not at time point 1 or time point 2. Thus, the increase in naïve T cells from baseline was higher for naïve CD4$^+$ T cells than naïve CD8$^+$ T cells at time point 1 (mean increase, 55 versus 24 cells/µl; $P < 0.05$) and at time point 2 (mean increase, 104 versus 68 cells/µl; $P < 0.05$). The additional changes between time points 1 and 2 in naïve T-cell numbers did not differ between naïve CD4$^+$ and naïve CD8$^+$ T cells.

Significant inverse Spearman rank correlations were observed between baseline naïve T-cell counts and the relative change (n-fold) in naïve T cells for both CD4$^+$ and CD8$^+$ T cells at time point 1 ($\rho = -0.53$, $P < 0.05$ for CD4; $\rho = -0.48$, $P < 0.05$ for CD8) and time point 2 ($\rho = -0.82$, $P < 0.01$ for CD4; $\rho = -0.64$, $P < 0.01$ for CD8).

Significant declines in Ki67 expression were observed in both CD4$^+$ and CD8$^+$ naïve and central memory T cells at time point 1 ($P < 0.01$) (Table 3). Before HAART, a median of 3.8% of naïve CD4 cells and 5% of naïve CD8 cells were Ki67$^+$, with the difference between the means reaching borderline statistical significance ($P = 0.05$). At time point 1 these numbers decreased to 1.7 and 2.3%, respectively ($P < 0.01$). The additional observed declines at time point 2 to 1.4 and 1.3%, respectively, were not statistically significant ($P > 0.05$, between time points 1 and 2). Moreover, the relative change (n-fold) in percent Ki67$^+$ naïve CD4$^+$ T cells between time point 0 to time point 1 inversely correlated with the corresponding relative change in CD4$^+$ naïve T-cell counts at the same time points (Spearman rank correlation, $\rho = -0.54$, $P = 0.017$). No similar correlation was observed between time points 1 and 2 or between any time points for naïve CD8$^+$ T cells.

Greater relative change (n-fold) in TRECs/µl than naïve T cells/µl in CD4$^+$ and CD8$^+$ T cells after HAART. A statistically significant increase in TRECs/µl from time point 0 to

TABLE 3. Flow cytometry parameters and thymic indices for group 1 patients during the study period

Parameter (n = 20)[a]	Value for the group at time point:[b]		
	0 (mean ± SD)	1 (mean ± SD)	2 (mean ± SD)
No. of CD4$^+$ (cells/μl)	255 ± 209	400 ± 296**	541 ± 264**
No. of CD4$^+$ RO$^-$ 27$^+$ (cells/μl)	87 ± 80	127 ± 140**	164 ± 132**
No. of CD4$^+$ RO$^+$ 27$^+$ (cells/μl)	129 ± 99	207 ± 128**	255 ± 120**
No. of CD4$^+$ RO$^+$ 27$^-$ (cells/μl)	59 ± 49	77 ± 67	82 ± 58**
CD4$^+$ RO$^-$ 27$^+$ (% Ki67$^+$)	4 ± 2	2 ± 1**	2 ± 1**
CD4$^+$ RO$^+$ 27$^+$ (% Ki67$^+$)	22 ± 9	12 ± 4**	11 ± 5**
CD4$^+$ RO$^+$ 27$^-$ (% Ki67$^+$)	16 ± 9	13 ± 6	9 ± 3**
No. of CD8$^+$ (cells/μl)	791 ± 390	672 ± 273	759 ± 238
No. of CD8$^+$ RO$^-$ 27$^+$ (cells/μl)	114 ± 54	138 ± 73	175 ± 89*
No. of CD8$^+$ RO$^+$ 27$^+$ (cells/μl)	369 ± 234	237 ± 115*	230 ± 93
No. of CD8$^+$ 27$^-$ (cells/μl)	357 ± 211	294 ± 229	297 ± 155
CD8$^+$ RO$^-$ 27$^+$ (% Ki67$^+$)	6 ± 2	2 ± 1**	2 ± 1**
CD8$^+$ RO$^+$ 27$^+$ (% Ki67$^+$)	21 ± 9	8 ± 3**	8 ± 4**
CD8$^+$ 27$^-$ (% Ki67$^+$)	29 ± 20	17 ± 7**	12 ± 5**
CT score	1.6 ± 0.8	1.9 ± 1	1.7 ± 1
Median (range)	1.5 (0.5–3.0)	1.5 (0.5–4.0)	1.5 (0.5–4.0)
Thymic volume (cm^3)	3.1 ± 3.1	3.9 ± 4.4	4.8 ± 5.1
Median (range)	1.6 (0.7–11.9)	1.7 (0.2–12.2)	1.9 (0.2–16.4)

[a] RO = CD45RO; 27 = CD27.
[b] *, $P < 0.05$; **, $P < 0.01$ (versus time point 0).

time point 1 ($P < 0.01$) was seen for both naïve CD4$^+$ T cells and naïve CD8$^+$ T cells (Fig. 1). A greater relative increase (n-fold) was seen in the number of TRECs/μl than in the naïve T-cell counts in both CD4$^+$ (median relative increases of 2.2- and 1.7-fold, respectively, $P < 0.01$) and CD8$^+$ T-cell pools (1.4- and 1.2-fold, respectively, $P < 0.01$) at time point 1. This observation is equivalent to an increase in the fraction of TRECs, i.e., TRECs per million naïve T cells, after initiation of HAART (Fig. 1), consistent with previously reported data (21, 32). The relative increase was observed to be lower for TRECs/μl than for naïve T-cell counts from time point 1 to time point 2, with this difference being statistically significant for CD4$^+$ T cells ($P < 0.05$) but not for CD8$^+$ T cells. No significant correlations were observed between the relative change in percent Ki67$^+$ naïve CD4$^+$ T cells at time point 1 to time point 0 and the relative change in TREC$^+$ cells at the same time points (for CD4$^+$, $n = 14$, $\rho = -0.03$, and $P = 0.47$; for CD8$^+$, $n = 16$, $\rho = -0.05$, and $P = 0.41$). The lack of correlation between changes in the proliferation of naïve T cells and the change in the fraction of TREC$^+$ cells per naïve T cell suggests that it is not the extent of reduction in proliferation per se that can explain the greater relative change in TRECs/μl versus naïve T-cell counts, but the latter does probably depend on changes in both the proliferation and disappearance rate of the naïve pool after the initiation of HAART.

Thymic indices did not significantly change during HAART and did not correlate with changes in TRECs. CT scoring was carried out by two radiologists blinded to clinical and laboratory data. The median baseline score was 1.5 (0.5 to 3.0). Median scores remained constant at time point 1 (1.5; range, 0.5 to 4) and at time point 2 (1.5; range, 0.5 to 4), with a median change for both time points of 0 (Table 3). When scores were clustered according to subjects with greater than and less than or equal to the median CD4 increase of 96 cells/μl at time point 1 and 257 cells/μl at time point 2, differences again were not significant. By volumetric analysis, the median score prior

to initiating HAART was 1.58 cm^3 (0.75 to 11.9). At time point 1 there was a median increase of 0.17 cm^3 (-4.24 to 9.33); an additional period of approximately 12 months of HAART resulted in a median change of -0.04 cm^3 (range, -5.27 to 6.5). None of these changes was statistically significant (Table 3). Baseline naïve CD4$^+$, but not naïve CD8$^+$, T-cell counts positively correlate with thymic volume (data not shown). An inverse statistically significant correlation was observed between the relative change (n-fold) in naïve T cell counts and baseline CT scores for naïve CD4$^+$ T cells ($\rho = -0.52$, $P < 0.05$) but not for naïve CD8$^+$ T cells ($\rho = -0.08$, $P > 0.05$). Partial Spearman rank correlation analysis that included age or baseline naïve T-cell counts did not qualitatively change the statistical significances of the above correlations. Similar patterns of correlations were observed when CT scores were replaced by thymic volumes. In addition, thymic volume changes did not correlate with changes in any TREC parameters.

Analysis of the model. A possible explanation for the greater relative change in TRECs/μl versus naïve T-cell counts can be provided by a model of naïve T-cell dynamics similar to the one described in equation 1 with the disappearance rate, d, a composite of two different factors: $d = \delta_A + \delta_R$, where δ_A represents the death rate of naïve cells and δ_R is the rate of priming of naïve cells into memory cells (7, 23). Assuming that the increase in the proliferation rate of naïve T cells during chronic HIV-1 infection ($p \rightarrow p + \Omega$) is mostly explained by the increase in the rate of priming of naïve T cells into memory cells ($\delta_R \rightarrow \delta_R + \Omega$) (Fig. 2A), naïve T-cell counts will not substantially change, since the increased recruitment of naïve T cells into memory cells is counterbalanced by the simultaneous increase in the proliferation rate of naïve T cells (without losing their phenotypes). Conversely, if the main effect of HAART consists of reducing the rate of priming ($\Omega \rightarrow 0$, with time), again, naïve T-cell counts will be only marginally affected since the consequent decrease in recruitment (and consequent loss) of naïve T cells is now counterbalanced by the simultaneous decrease in the proliferation rate. However, the reduction in
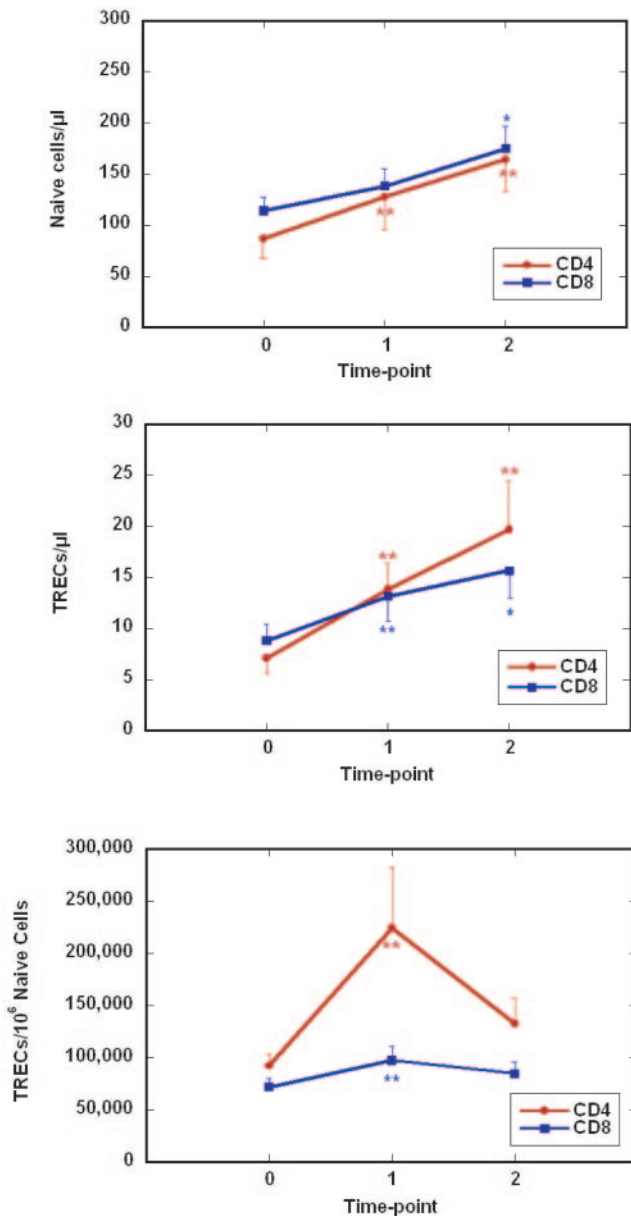
FIG. 1. Naïve T-cell counts, TRECs per microliter of blood, and fraction of TREC$^+$ T cells per 10^6 naïve T cells for CD4 and CD8$^+$ T cells at the three time points of the study. Values are the means ± standard errors of the means. ∗, $P < 0.05$; ∗∗, $P < 0.01$ (versus time point 0).

proliferation will lead to an increase of the naïve T-cell (and thus TREC$^+$ T cell) life span or, equivalently, the decrease in disappearance rate d. This leads to an increase in TREC$^+$ T cells/μl, and since the naïve T-cell counts are only marginally affected, the fraction of TREC$^+$ T cells per naïve T cell is also predicted to increase.

With σ values of >0 this generalization predicts a greater decrease in the proliferation than the disappearance rate when a new quasi-steady state is reached following initiation of HAART (data not shown). The dynamics of naïve CD8$^+$ T cells observed in this study are consistent with this: a significant increase in the number of TREC$^+$ T cells/μl is accompanied by

a limited increase in naïve T-cell counts after initiation of HAART (Table 3 and Fig. 1 and 2B). This model also predicts that the decrease in the proliferation rate after HAART is not expected to correlate with the increase in naïve T-cell counts, since the latter will be only marginally affected regardless of the rate at which naïve T-cell proliferation normalizes. Again, our data are consistent with this: there is no significant correlation between the increase of CD8$^+$ naïve T-cell counts and the decrease of percent Ki67$^+$ CD8$^+$ naïve T cells between time points 0 and 1 or later.

For CD4$^+$ naïve T cells, the above model is inadequate to explain the observed dynamics. However, the transient increase in the fraction of TREC$^+$ T cells per naïve T cell and the following new steady state reached after 18 months of antiretroviral therapy can be explained by assuming that the death rate, δ_A, is also enhanced ($\delta_A \rightarrow \delta_A + \Psi$) during HIV-1 infection due to activation-induced cell death. The effect of HAART is again to normalize the proliferation rate and the disappearance rate of the CD4$^+$ naïve T cells. However, the latter now includes both the death rate and the priming rate, which demonstrate differential dynamics related to changes in Ω and Ψ, respectively. A HAART-induced normalization of the death rate that is approximately 10-fold slower than the normalization of the proliferation rate adequately accounts for the changes in both TREC fractions and TREC$^+$ T cells/μl that are observed for CD4$^+$ naïve T cells (Fig. 2C).

Previous reports have identified a positive correlation between baseline percentages of proliferating and apoptotic (terminal deoxynucleotidyltransferase-mediated UTP nick end labeling-positive cells) T cells (36). If the HIV-1-induced increases in proliferation and death rates of naïve T cells (Ω and Ψ) are similarly proportionally related, then the model also predicts an inverse correlation between the HAART-induced decrease in proliferation of naïve T cells and the recovery of naïve T-cell counts (data not shown) which we observed for CD4$^+$ naïve T cells. Thus, HIV-1-infected patients with higher proliferation and death rates of naïve T cells during chronic infection would demonstrate a faster recovery of naïve T-cell counts after HAART, with most of the recovery explained by normalization of the death rate rather than by normalization of the proliferation rate, which, as in the earlier model, is still counterbalanced by the simultaneous reduction in the rate of priming.

Kinetics of BrdU-labeled naïve T cells prior to and after HAART. To further evaluate the effects of HAART on the proliferation and disappearance rates of naïve T cells, we analyzed the kinetics of BrdU-labeled naïve T cells prior to and after initiation of HAART in six HIV-1-infected patients. Figure 3 shows the theoretical curves obtained by best fitting equation 3 to the fraction of BrdU-labeled naïve CD4$^+$ T cells for each patient, before and after initiation of therapy. Because the percentages of BrdU-labeled cells within the naïve T-cell population are relatively small, and thus closer to the detection threshold, this analysis resulted in a weak convergence of the nonlinear best-fitting procedure using equation 3. Thus, equation 4 was used to estimate the slope s from the time of infusion to the peak of labeling, and a single exponential decay function has been used to estimate d^* from the time of peak labeling. As shown in Table 4, this modeling predicts a decay in s greater than a decay in d^* for naïve CD4$^+$ T cells after
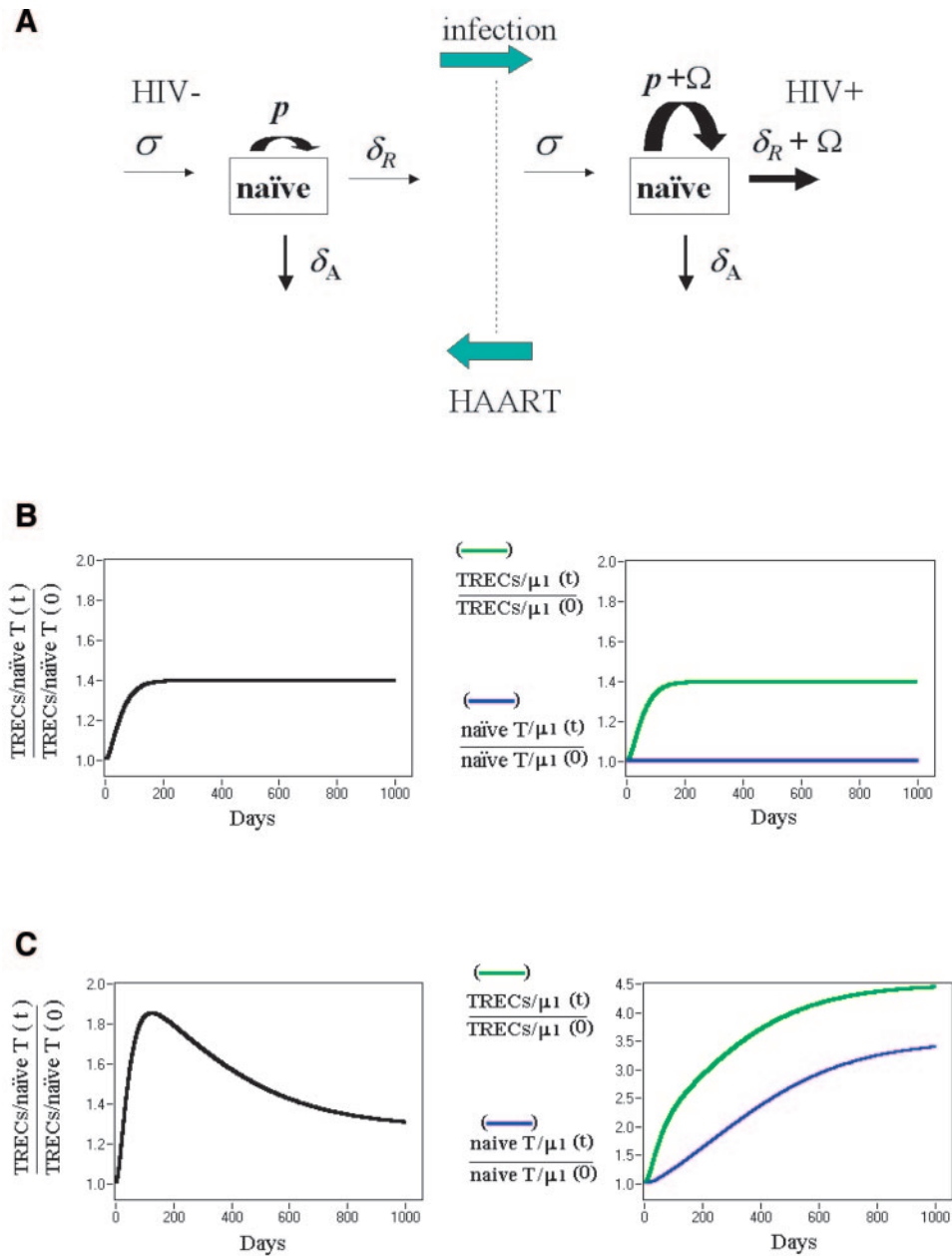
FIG. 2. (A) Diagram of the model used to describe the changes in naïve T-cell counts and TREC$^+$ T cells/µl during HIV infection and after initiation of HAART. The proliferation rate of naïve T cells is increased during HIV-1 infection by a factor Ω as a result of the increase in the rate of priming into memory cells. (B) HAART-induced changes in the fraction of TREC$^+$ T cells per naïve T cell (left) and TREC$^+$ cells/µl and naïve T-cell counts (right) predicted by a model that assumes that the HIV-1-induced increase in proliferation of naïve T cells is largely explained by the increase in rate of priming: $\frac{dT}{dt} = \sigma + [p + \Omega(t)]T - [\delta_A + \delta_R + \Omega(t)]T$ and $\frac{dT^+}{dt} = f\sigma - [\delta_A + \delta_R + \Omega(t)]T^+$, where $\sigma = 1$ cell $\cdot$ µl^{-1} $\cdot$ day^{-1}, $f = 0.1$, $p = 0.01$ day^{-1}, and $d = \delta_A + \delta_R = 0.03$ day^{-1}. Here, $\Omega(t)$ is modeled as a single exponential decaying function from the administration of HAART: $\Omega(t) = \Omega_0 e^{-\lambda t}$, with $\Omega_0 = 0.02$ day^{-1} and $\lambda = 0.05$ day^{-1}. The graphs show the ratio of the value of the individual parameters at time t to the value at time zero. The dynamics predicted by this model are similar to the dynamics observed for CD8$^+$ naïve T cells in this study. (C) HAART-induced changes for the same variables when the death rate, δ_R, is also enhanced by a factor $\Psi(t)$ during chronic HIV-1 infection due to activation-induced cell death. Here, $\Psi(t)$ is modeled as a single exponential decaying function from the administration of HAART: $\Psi(t) = \Psi_0 e^{-\gamma t}$, with $\Psi_0 = 0.025$ day^{-1} and $\gamma = 0.005$ day^{-1}. Other parameters are as follows: $\Omega_0 = 0.045$ day^{-1}, $\lambda = 0.05$ day^{-1}, $\sigma = 1$ cell $\cdot$ µl^{-1} $\cdot$ day^{-1}, $f = 0.1$, $p = 0.01$ day^{-1}, and $d = \delta_A + \delta_R = 0.02$ day^{-1}. The dynamics predicted by this model are similar to the dynamics observed for CD4$^+$ naïve T cells in this study.
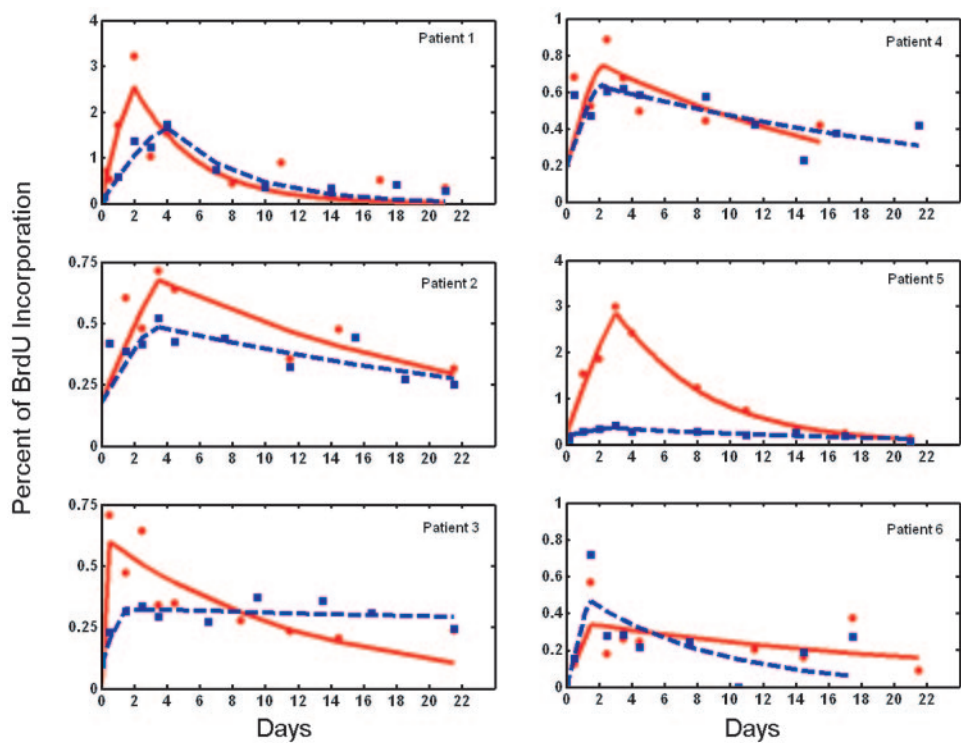
FIG. 3. Comparison of experimental data with modeling of the kinetics of BrdU incorporation and decay by $CD4^+$ naïve T cells for each patient. Lines represent the modeling, and symbols represent the actual data points (red, pre-HAART; blue, post-HAART).

initiation of HAART (median relative decrease [n-fold] in s of 2.3 and median relative decrease in d^* of 1.5; $P = 0.03$). Since changes in s imply changes in mean proliferation rates, a greater decay in s than d^* implies a more dramatic decrease in proliferation than disappearance rates of naïve T cells after HAART, whether d^* represents the apoptotic death rate plus the rate of priming into memory T cells or the difference between the disappearance rate in the blood, d, and the proliferation rate in the blood, p (data not shown).

DISCUSSION

In this study we have analyzed the relationship between T-cell turnover, thymic function, and immune activation in HIV-1-infected patients, focusing on naïve $CD4^+$ and naïve $CD8^+$ T cells, to better understand the contribution of these various parameters to the immunologic changes seen during HIV infection and therapy. We specifically targeted a broad range of baseline viral load and $CD4^+$ T-cell counts in order to highlight the dynamics across the spectrum of HIV infection. At baseline, naïve T-cell numbers were lower in the CD4 pool compared to the CD8 pool, but no difference between the two groups was observed in the percentages of proliferating naïve T cells or in the number of TRECs. Even though a dramatic decrease in proliferation is observed for both naïve $CD4^+$ and $CD8^+$ T cells after the first 6 months of therapy, we found that naïve $CD4^+$ T-cell numbers significantly increased after initiation of HAART, but naïve $CD8^+$ T-cell numbers were only marginally affected. Baseline naïve T-cell counts inversely correlated with the relative changes in naïve T cells in both subpopulations of cells, and TRECs/µl increased in both subsets

TABLE 4. Parameter estimates for the dynamics of BrdU-labeled naïve $CD4^+$ T cells before and after HAART in group 2 patients

Patient no. ($n = 6$)	Source and disappearance rates before and after HAART[a]			
	s_0 day^{-1} (95% CI)	s_1 day^{-1} (95% CI)	d^*_0 day^{-1} (95% CI)	d^*_1 day^{-1} (95% CI)
1	1.51 (1.34, 1.76)	0.65 (0.59, 0.72)	0.10 (0.04, 0.15)	0.09 (0.06, 0.12)
2	0.13 (0.06, 0.21)	0.09 (0.03, 0.14)	0.04 (0.02, 0.06)	0.03 (0.02, 0.05)
3	1.21 (NA)	0.09 (0.05, 0.13)	0.05 (0.03, 0.08)	0.01 (−0.01, 0.02)
4	0.24 (0.07, 0.38)	0.11 (0.02, 0.19)	0.05 (0.02, 0.07)	0.03 (0.00, 0.06)
5	0.96 (0.80, 1.07)	0.11 (0.08, 0.12)	0.18 (0.16, 0.20)	0.04 (0.02, 0.07)
6	0.33 (0.25, 0.43)	0.43 (0.34, 0.55)	0.01 (−0.04, 0.08)	0.04 (0.00, 0.09)
Mean for group	0.73 ± 0.52	0.23 ± 0.21	0.07 ± 0.06	0.04 ± 0.02

[a] Shown are the means ± SDs of the calculated source and disappearance rates before (s_0 and d^*_0) and after (s_1 and d^*_1) initiation of HAART. CI, confidence interval; NA, not available.

of cells. We also observed an increase in the fraction of TREC$^+$ T cells per naïve T cell, which is equivalent to a faster growth of TREC$^+$ T cells versus total naïve T cells, for both CD4 and CD8 cells following initiation of HAART. We did not observe significant changes of thymic volumes during time or statistically significant correlations between changes in thymic volumes and changes in naïve T-cell numbers or TRECs/µl.

To provide a unified description of the concomitant changes in naïve T cells (TRECs/µl and the percentage of proliferating naïve T cells during HAART) that can explain the observed differences in the dynamics of these variables in the CD4 and CD8 subpopulations of T lymphocytes, we developed a mathematical model based on a generalization of a model originally described by Hazenberg et al. (21). We have generalized the disappearance rate of naïve T cells as the sum of the rates of naïve cells priming into memory cells and naïve cell death and assumed that the increase in proliferation of naïve T cells during chronic infection is primarily explained by the increase in the rate of priming of naïve T cells into memory cells. This simple theoretical framework is sufficient to predict the simultaneous increases in the fraction of TREC$^+$ T cells per naïve T cell and the number of TREC$^+$ T cells in the periphery after initiation of HAART. The model explains the dynamics of naïve CD8$^+$, but not CD4$^+$, T cells after institution of HAART. To explain the dynamics of naïve CD4$^+$ T cells, we postulate that there is an increase in the apoptotic death rate of naïve T cells during HIV-1 infection related to immune activation or to the increase in proliferation rate and that there is delayed normalization of the apoptotic death rate compared to the proliferation rate, as has been reported for total CD4$^+$ T cells in lymph node samples of HIV-1-infected patients before and after initiation of HAART (48). This model does not require (or exclude) changes in thymic output or redistribution of T lymphocytes from the lymphoid tissue as additional mechanisms contributing to naïve T-cell recovery.

The normalization of the death rates can also account for the inverse correlation between baseline naïve T-cell counts and the relative change in naïve T-cell counts after initiation of HAART. However, the increase in naïve T cells appears to be lower for naïve CD8 T cells than for the naïve CD4$^+$ T cells, which suggests independent mechanisms of peripheral normalization for the different populations. This dichotomy, observed in the dynamics of naïve CD4$^+$ T cells compared to naïve CD8$^+$ T cells after HAART, as well as the presence of an inverse correlation between baseline thymic scores and the relative change (n-fold) in naïve T-cell numbers for CD4$^+$ but not CD8$^+$ T cells is difficult to explain solely as a result of changes in thymic output rates or trafficking effects, since these should not have differential effects on the two populations of naïve T cells. Moreover, based on this model, the observed inverse correlation between baseline counts and the relative change in naïve T-cell counts after HAART for both naïve CD4$^+$ and naïve CD8$^+$ T cells suggests that increases in the death rate affect both populations. However, the greater baseline depletion of naïve CD4$^+$ T cells compared to naïve CD8$^+$ T cells, together with the concomitant increase in TRECs per microliter in both compartments following therapy, suggests, as highlighted by the model, that similar mechanisms drive both naïve CD4$^+$ and naïve CD8$^+$ T cells to be primed into memory cells, but for unknown reasons the increase in the

death rate is more pronounced in the CD4$^+$ than in the CD8$^+$ naïve T-cell populations. Interestingly, Li et al. have recently shown that, at least in the settings of acute simian immunodeficiency virus infection, higher levels of Fas- and Fas ligand-mediated apoptosis are observed within CD4$^+$ but not CD8$^+$ T lymphocytes in the lamina propria, which may result from massive exposure of CD4$^+$ T cells to virion gp120 (33). Our data suggest that during chronic infection, the differential ability to tolerate similar increases in proliferation, Ω, is an intrinsic property of each subpopulation of naïve T cells. An alternative scenario in which Ω is different between the two subpopulations would require that naïve CD4 T cells have a higher proliferation rate than naïve CD8 T cells to account for the relative loss in naïve CD4 T cells. However, this was not observed in our data when we looked at the baseline fractions of proliferating naïve CD4 and CD8 T cells.

It is important to note that this model represents an idealized situation and that deviations from this model, resulting, for instance, from the presence of nonlinear contributions of trafficking of lymphocytes or of replenishment of the peripheral pool by thymic output, might result in a situation that is far from the quasi-steady-state condition assumed in equation 1. In the latter circumstances, TREC content after initiation of HAART can potentially be affected in an unpredictable manner.

The hypothesis of a more pronounced decrease in the proliferation rate than the disappearance rate of naïve T cells after HAART is supported by the kinetics of BrdU-labeled naïve T cells studied longitudinally (pre- and post-HAART) that we observed in a smaller group of HIV-1-infected patients.

In principle, changes in the fraction of naïve T cells carrying TRECs upon exiting the thymus (f in equation 1) might also explain a greater relative change in TREC$^+$ T cells/µl than naïve T-cell counts after initiation of HAART (32). Among the four parameters discussed in this analysis (d, p, σ, and f), f is the least investigated. Dion and colleagues (8) have recently shown an increase of the ratio α-TRECs/β-TRECs after initiation of HAART, which suggests that the newly produced naïve T cells undergo more intrathymic divisions before entering the peripheral pool. Since β-TRECs are produced before α-TRECs, this would lead to a decrease, not an increase, in f after initiation of HAART, thus excluding changes in f as a major factor affecting the dynamics of the fraction of TREC$^+$ T cells after initiation of HAART.

This analysis provides evidence that changes in peripheral proliferation and disappearance rates of naïve T cells, rather than changes in thymic output, explain the observed dynamics of TRECs and the fraction of proliferating T cells during HAART. But what is the mechanism that drives naïve T cells to proliferate faster during chronic infection? The first pathogenic effect of HIV-1 infection might consist of an increase in the rate of priming of naïve T cells due to a generalized state of chronic immune activation. In this scenario, the HIV-1-induced increase in the proliferation rate could serve as a compensatory (homeostatic) mechanism aimed at maintaining the naïve T-cell count constant, in response to the loss of naïve T cells that have been primed into memory cells. Alternatively, the $p(t)$ expression of the proliferation rate of our model could also be modeled as a function of the number of cells, $T(t)$, for instance, following a density-dependent law (10). Under this scenario, changes in thymic output and consequent (homeo-

static) changes in peripheral proliferation of naïve T cells could also explain the observed TREC dynamics, as suggested by Dutilh et al. to explain the changes of TREC content during aging (10). However, given the relatively short time frame (a few weeks) in which significant changes of proliferation and disappearance rates are seen (36) and the negligible contribution by the thymus seen in thymectomy studies (as recently described for nonhuman primates by Arron et al. [1]), as well as our thymic CT data, we feel that peripheral mechanisms (including homeostatic mechanisms) leading to changes in proliferation and disappearance rates are the major factors affecting the dynamics of TRECs. The observed dichotomy in the dynamics of naïve $CD4^+$ and naïve $CD8^+$ T cells is difficult to explain by invoking changes in thymic output as the sole mechanism responsible for changes in peripheral (homeostatic) proliferation rates of naïve T cells.

If a homeostatic mechanism governs the proliferation of naïve T cells, we would expect, as has been previously suggested for the entire population of T cells (24), an inverse correlation between relative change (n-fold) in naïve T-cell counts after HAART and the relative changes in the percentages of proliferating naïve T cells. In our study we do observe such an inverse correlation for naïve $CD4^+$ but not naïve $CD8^+$ T cells. Alternatively, the first effect of activation might consist of inducing naïve T cells to proliferate faster without losing their phenotype (45–47), bringing these cells closer to the priming activation threshold which leads to an increased disappearance rate of naïve T cells. In this scenario, the increase in the proliferation rate is primarily explained by the increase in the rate of priming during chronic infection. The simultaneous decrease in both the proliferation and priming rates after initiation of HAART should generate a lack of correlation between the recovery of naïve T-cell counts (only marginally affected) and the decrease in the percentage of proliferating naïve T cells. This latter paradigm would explain the observed lack of such correlation for naïve $CD8^+$ T cells. The additional assumption that higher levels of proliferation are associated with higher levels of apoptosis is required to explain the presence of this inverse correlation for $CD4^+$ naïve T cells. Both scenarios show consistency with a differential change in the proliferation and disappearance rates of naïve T-cells. Based on current available data, it is difficult to make conclusive arguments in support of either hypothesis. Thus, further investigations are required to clarify the mechanisms responsible for the increased proliferation of naïve T cells induced by HIV-1.

ACKNOWLEDGMENTS

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